AVIAN DISEASES 51:590–593, 2007

Research Note—

Induction of Tibial Dyschondroplasia by Carbamate and Thiocarbamate Pesticides

N. C. Rath,[A] W. E. Huff, G. R. Huff, and L. Kannan

Poultry Production and Product Safety Research Unit, Agricultural Research Service, USDA, University of Arkansas, Fayetteville, AR 72701

Received 26 September 2006; Accepted 1 December 2006

SUMMARY. Tibial dyschondroplasia (TD) is a major poultry leg problem, the natural etiology of which is unknown. Certain dithiocarbamate pesticides such as tetramethyl thiuram disulfide (thiram) have been shown to induce the disease in chickens. Because many different carbamate and thiocarbamate chemicals are used in a number of agricultural, industrial, and household applications, the objective of this study was to determine whether all chemicals of these categories induce TD and whether there is a concentration-dependent relationship between the ingestion of these chemicals and the incidences and the severity of the disease. Week-old broiler chicks were fed diets containing thiram or other assorted carbamate and thiocarbamate pesticides mixed in feed for 24–48 hr between ages 8 and 10 days. The birds were killed on day 15 and the proximal tibial and tarsometatarsal growth plates were evaluated for the presence and severity of TD lesions. TD was distinguished by broadening of growth plates; upon histologic exam chondrocytes appeared to be shrunken and dead. When compared by including equimolar concentrations of these chemicals in the feed, the dithiocarbamates with more than two sulfide groups, such as disulfiram, ferbam, thiram, and ziram were potent inducers of TD, whereas those with two sulfides to no sulfide group appeared ineffective at inducing TD. Both thiram and ferbam also reduced the bird's body weights. Thiram increased the incidence and the severity of the disease, denoted by TD index, in a dose-dependent manner. These results suggest that inadvertent contamination of feed or litter with some of these or similar chemicals may cause leg problems in poultry.

RESUMEN. *Nota de Investigación*—Inducción de discondroplasia tibial por los pesticidas carbamatos y tiocarbamatos.

La discondroplasia tibial es el principal problema de las patas en aves domésticas, cuya etiología se desconoce. Se ha demostrado que ciertos pesticidas ditiocarbamatos tales como el tetrametil tiuram disulfito (thiram), inducen la enfermedad en pollos. Debido a que diferentes carbamatos y tiocarbamatos químicos son usados en grandes cantidades en aplicaciones agrícolas, industriales y domésticas; el objetivo de este estudio fue determinar si todos los químicos de esta categoría inducen discondroplasia tibial y si existe una relación dependiente de la concentración entre la ingestión de estos químicos y la incidencia y severidad de la enfermedad. Pollos de engorde de una semana de edad fueron alimentados por 24 a 48 horas, entre las edades de 8 y 10 días, con dietas que contenían thiram u otros pesticidas carbamatos y tiocarbamatos mezclados con el alimento. Las aves fueron sacrificadas en el día 15 y se evaluaron las placas de crecimiento de la tibia proximal y el tarsometatarso para identificar la presencia y la severidad de lesiones de discondroplasia tibial. La discondroplasia tibial fue identificada por el aumento en la placas de crecimiento; al examen histológico los condrocitos parecían estar disminuidos de tamaño y muertos. Cuando se realizó la comparación según la concentración equimolar de estos químicos incluidos en el alimento, los ditiocarbamatos con más de dos grupos sulfuro tales como disulfiram, ferbam, thiram, and ziram; fueron inductores potentes de discondroplasia, mientras que aquellos con dos grupos sulfuro o sin éstos, no fueron efectivos en la inducción de esta lesión. Tanto el thiram como el ferbam también redujeron el peso corporal de las aves. El thiram incrementó la incidencia y la severidad de la enfermedad, caracterizada por el aumento en el índice de discondroplasia tibial de una manera dosis dependiente. Estos resultados sugieren que contaminaciones que pasan inadvertidas en el alimento o en la cama, con estos químicos o productos similares, pueden causar problemas en las patas de las aves de corral.

Key words: dithiocarbamates, tibial dyschondroplasia, chicken, growth plate

Abbreviations: ETU = ethylenethiourea; TD = tibial dyschondroplasia

Carbamates and dithiocarbamates are organic compounds widely used in a variety of agricultural and household applications as herbicides, fungicides, anthelmintics, insecticides, pest repellents, and bacteriostatic agents (2,4,5,9,10). The carbamates are represented by a general structure R-NH(CO)O^-, and the dithiocarbamates are characterized by R-NH(CS)S^-, with R representing an alkyl group (27). The fungicide and herbicide actions of carbamates are attributed to their ability to inhibit vital processes that regulate fungus and seedling growth and metabolism. As insecticides and pesticides these chemicals impair and damage nerve functions (28). Many different carbamates, thiocarbamates, and their derivatives are used for various agricultural applications. Carbaryl, also commercially known as Sevin™, is a carbamate pesticide that is used as an insecticide for home gardens, commercial agriculture, forestry, and rangeland applications (11). Dithiocarbamates, though primarily used as fungicides and pest repellents, are also employed in industrial applications to vulcanize rubber, and as biocides in paper manufacturing (4,9,10). Some dithiocarbamates are used in the treatment of wastewater to complex heavy metals, whereas others, for example disulfiram, have been used in clinical medicine to treat alcoholism (2). Although these chemicals have been useful in improving agriculture, some accidental overexposure has been known to cause metabolic dysfunctions, neurotoxicity, and endocrine disruption (4,25). Chronic exposure to thiram (tetramethyl thiuram disulfide) increases leg problems in chickens, causing tibial dyschondroplasia (TD) by inducing incomplete ossification at the proximal growth plates of the tibial and tibiotarsal bones

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[A]Corresponding author. E-mail: nrath@uark.edu

Table 1. Common and chemical names (parentheses) of assorted carbamates and dithiocarbamates and their uses. Table is based on references 4, 5, 9, and 10 and the chemical data sheets provided by the manufacturers.

Chemicals	Formula	Uses
Carbaryl (1-naphthyl methylcarbamate)	$C_{12}H_{11}NO_2$	Insecticide, acaricide, plant growth inhibitor
Disulfiram (tetraethyl thiuram disulfide)	$C_{10}H_{20}N_2S_4$	Fungicide, acaricide, treatment of alcoholism
Ethylenethiourea	$C_3H_6N_2S$	Degradation product of ethylenebisdithiocarbamate, also used in the vulcanization of rubber
Ferbam (ferric dimethyldithiocarbamate)	$C_9H_{18}N_3S_6Fe$	Fungicide, used in wood treatment
Potassium dimethyl dithiocarbamate	$C_3H_6NS_2K$	Heavy metal precipitator, rubber accelerator, biocide, fungicide
Sodium metam (methyldithiocarbamate sodium)	$C_2H_4NS_2Na$	Microbiocide, algaecide, anthelmintic
Thiram (tetramethyl thiuram disulfide)	$C_6H_{12}N_2S_4$	Fungicide, a general-use pesticide, bacteriostatic soap additive, bird repellant
Ziram (zinc bis[dimethyldithiocarbamate])	$C_6H_{12}N_2S_4Zn$	Fumigant, herbicide, fungicide

(3,7,13,18,29,30). We have shown that including thiram at doses of 50 or 100 ppm in the feed for a day or two during posthatch development can increase the incidence and severity of TD in chickens (19,22). Because some of these chemicals can occur as contaminants in products that are used for litter materials, such as wood shavings, peanut hulls, and paper products; as chemical sprays to control beetles and flies; and as rodent repellants, the objective of this study was to determine the ability of different carbamate and thiocarbamate pesticides to induce TD in broiler chickens, and to check the dose relationship of any effect observed.

MATERIALS AND METHODS

Chemicals. Thiram, disulfiram, and carbaryl were purchased from Sigma Chemical Company (St. Louis, MO). Ethylenethiourea was obtained from Spectrum Chemical Company (Tucson, AZ). Ferbam, ziram, sodium metam, and K-dithiocarbamate were donated by Taminco N. V. (Ghent, Belgium). The chemical names and some of their uses are shown in Table 1.

Induction of TD. Two experiments were conducted to determine 1) whether all carbamate and dithiocarbamate pesticides are capable of inducing TD in chickens and 2) whether there exists a dose-dependent relationship between the quantity of the chemicals consumed and the incidence and severity of TD. The dose-relation experiment used thiram as the model chemical. Day-old male broiler chicks were raised in Petersime batteries at a density of 10–12 birds/cage under a light period of 23 hr and given corn–soybean-based broiler starter diet prepared according to National Research Council specifications (17), and water *ad libitum*. On day 7, feed was withdrawn overnight for a period of 14 hr following which the birds were given diets containing different carbamate and thiocarbamate pesticides and a thiocarbamate metabolite, ethylenethiourea (8,16). The chemicals were mixed at a concentration of 0.43 mmol/kg feed except for ethylenethiourea, which was used at 0.86 mmol/kg. The molar concentration of carbamates and dithiocarbamates was based on an equivalency of 100 ppm thiram, which was shown to significantly increase TD index following 2 days of feeding trials (19,22). Control chickens received diets without any chemicals. The dose-dependent effect was determined using only thiram; the birds received diets containing the pesticide at concentrations ranging from 0 to 100 ppm. The experiments with different chemicals consisted of 1 or 2 days of feeding trials with one set of birds receiving diets for 1 day whereas the other received the diets for 2 days. The experiments were carried out at different times and repeated twice. After scheduled feeding of diets containing different chemicals for 1 or 2 days, all chickens were returned to their regular control diets until necropsy on day 15. Individual body weight of birds were recorded prior to scheduled feeding and then prior to necropsy on day 15. Total feed consumption per group of birds in 2 days of feeding was determined to estimate the approximate amount of chemicals consumed per bird per day. The chickens were killed by cervical dislocation and the incidence and severity of TD were determined by gross examination of the proximal growth plates of both right and left legs. Some growth plates were also examined histologically.

Growth plate evaluation. Sagittal sections of proximal growth plates of tibia and tarsometatarsus were examined for the incidence and severity of TD lesions as described previously (19). Only tibial lesions were scored. An arbitrary score of 0 to 2 was applied based on the width of growth plate between proximal to distal aspect with 0 = normal, 1 = width broadened to about twice the normal size, 2 = a severe lesion with the growth plate widened beyond twice its normal size (19). The TD index was calculated by multiplying incidence times severity. In trials determining the efficacy of chemicals to cause TD, sections of growth plates from the left tibia from 15 birds in each group were fixed in neutral buffered formalin, then digitally scanned and photographed using a Hewlett-Packard Scanjet 4C; the growth plate widths were measured using Image ProPlus software (Media Cybernatics, Silver Spring, MD). Each growth plate was measured at three or four positions, gathering between 50–55 data points per group.

Histology. Growth plates from four birds per group were decalcified and processed for histology, stained with hematoxylin and eosin, and examined microscopically.

Statistics. Quantitative data were expressed as mean ± SEM, analyzed by general linear model (GLM) procedure, and separated using

Table 2. The effects of different carbamate and dithiocarbamate pesticides and ethylenethiourea on the body weight and TD indices of 15-day-old chickens fed for 1 or 2 days with the chemicals. Values in a column with different superscripts represent $P < 0.005$. ND = not determined.

Treatment	1-day treatment ($n = 18$)		2-day treatment ($n = 24$)	
	Body weight (g)	TD index	Body weight (g)	TD index
Control	430 ± 8	0.04 ± 0.04^{d}	465 ± 13^{a,b}	0 ± 0^{d}
Carbaryl	ND	ND	474 ± 14^{a,b}	0 ± 0^{d}
Disulfiram	426 ± 9	0.30 ± 0.07^{b}	479 ± 13^{a,b}	0.65 ± 0.03^{c}
Ethylenethiourea	418 ± 11	0.09 ± 0.08^{d}	476 ± 12^{a,b}	0 ± 0^{d}
Ferbam	422 ± 9	0.79 ± 0.17^{a}	406 ± 9^{c}	1.92 ± 0.08^{a}
K-dimethyl dithiocarbamate	425 ± 17	0.17 ± 0.11^{d}	468 ± 15^{a}	0.08 ± 0.07^{d}
Metam sodium	416 ± 10	0.21 ± 0.11^{b,c}	456 ± 10^{b}	0.07 ± 0.07^{d}
Thiram	413 ± 10	0.67 ± 0.16^{a}	391 ± 10^{c}	1.85 ± 0.15^{a}
Ziram	437 ± 7	0.39 ± 0.11^{b}	474 ± 13^{a,b}	1.08 ± 0.24^{b}

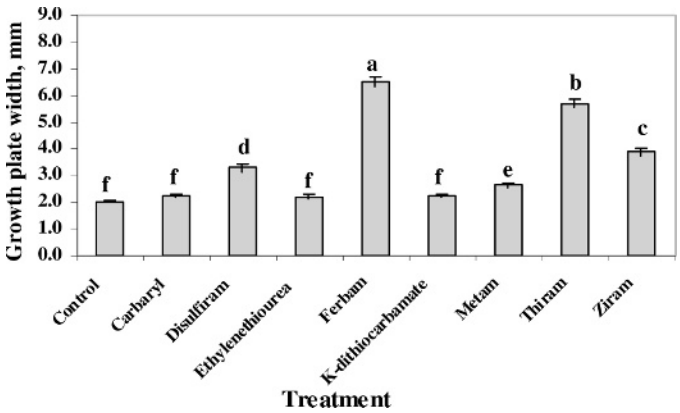

Fig. 1. Growth plate width (mm) of birds fed diets containing different carbamates and dithiocarbamates for 2 days. Columns with different superscripts are significantly different from each other ($P \leq 0.05$).

Duncan's multiple range tests with SAS statistical software (23). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results of the experiments comparing the effect of different carbamates and dithiocarbamates to induce TD are shown in Table 2 and Figs. 1, 2. Whereas carbaryl, potassium dithiocarbamate, sodium metam, and ethylenethiourea were least effective in inducing TD, the dithiocarbamates with more than two sulfide groups invariably induced the disease in the proximal growth plates of both tibia and tibiotarsal bones with either 1 or 2 days of treatment (Table 2; Fig. 1). Both thiram and ferbam caused the highest incidences of TD, followed by ziram and disulfiram. Thiram and ferbam also reduced the body weights of the birds with 2 days of feeding although the pretreatment body weights of birds in all groups were similar (data not shown). On an average, the approximate chemical consumption was calculated to be 28 ± 1 μmol/ kg/day. In the experiment determining the dose-dependent effect of thiram on the incidence and severity of TD, the results showed a linear increase in the TD index with 20–80 ppm of the chemical, reaching to the highest level at 100 ppm (Fig. 3). Several past studies by different investigators have shown that chronic feeding of chickens during the posthatch period with thiram at levels of 25–60 ppm in the feed induces TD (3,13,18,30). However, our study shows that even a short-term exposure of 1 or 2 days to selective dithiocarbamate pesticides may be enough to increase the

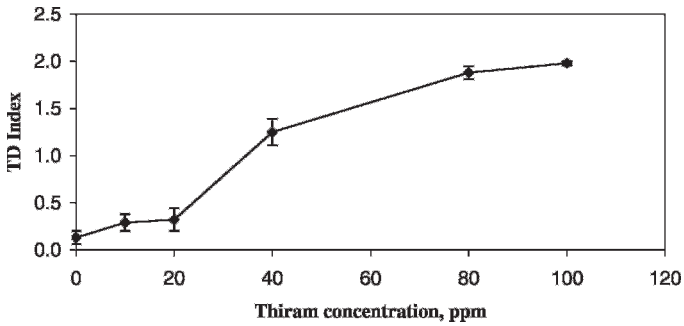

Fig. 3. Concentration-dependent increase in TD index with thiram.

incidence of TD with potential to cause severe TD in nearly 100% of birds, implying that these chemicals in small concentrations can cause sustained damage. All TD lesions induced by these chemicals had typical histology with condensed chondrocytes in the hypertrophic regions of the growth plate and empty lacunae, reminiscent of cell death (Fig. 2) (21,22,26).

The relationship between the sulfide content of dithiocarbamates and the pathogenesis of TD is not clear. Whether severity effect is because of their longer tissue retention or because of higher toxicity is not known. One of the major effects of dithiocarbamates is their ability to complex metal ions. It has been suggested that this contributes to their TD-inducing effects (13,18), although in a previous study we did not observe thiram to affect blood calcium, iron, copper, or magnesium concentrations following 2 days of feeding. Thiram did, however, effectively induce TD in more than 85% of birds (19). Dithiocarbamates have also been noted for their ability to interact with proteins, enzymes, and cofactors including glutathione, forming mixed disulfides or altering cellular 'redox' states. These changes have been postulated to interfere with cellular functions and affect tissue viabilities (1,5,6,14). Studies *in vivo* showed thiram to cause depletions of growth-plate glutathione concentration and induce chondrocyte and vascular cell death (22), an effect that was also noticed in an *in vitro* study using chondrocyte culture (20). Other biological effects of dithiocarbamates include their ability to modulate nuclear factor κB (24) and proteosome activity (30), both of which are likely to affect cell physiology. Although it is possible that many diverse actions of dithiocarbamates may contribute to their neurotoxic, antiadrenergic, and antiangiogenic activities (12,15,25,31) these data are at best derived from studies with very few representative chemicals such as thiram and disulfiram. Some studies have suggested that complex dithiocarbamates are metabolized to their corresponding thiols and dithiocarbamic acids, which may be responsible for their biological activities

Fig. 2. Growth plate morphology and histology of hypertrophic zone cartilage from 15-day-old chickens fed a control diet (a, b) or diets containing ferbam (c, d) for 2 days between days 7 and 9.

(5). However, neither sodium or potassium dithiocarbamate nor ethylenethiourea, a major metabolite of ethylenebisdithiocarbamate (8,16), were able to induce TD either in the current study or in a previous study (19), although it is not known whether these simpler chemicals would have similar effects following long-term treatments.

In conclusion, this study shows that complex dithiocarbamate pesticides with more than two disulfide groups are likely to cause poultry leg problems. Judicious use of such pesticides, specifically around poultry houses, may lower the incidence of tibial dyschondroplasia and other leg problems affecting young poultry.

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ACKNOWLEDGMENTS

Thanks are due to David Horlick, Dana Bassi, Sonia Tsai, Scott Zornes, and Wally McDonner for technical assistance.